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Effects of Kepone (Trade Name) on the Sheepshead Minnow in an Entire Life-cycle Toxicity Test

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16. ABSTRACT

An entire life-cycle toxicity test with the estuarine sheepshead minnow (Cyprinodon variegatus) and the organochlorine insecticide Kepone in seawater showed that the maximum acceptable toxicant concentration (MATC) lies between 0.074 and 0.12 µg Kepone/ℓ, based on reduced standard length of progeny at 0.12 µg/ℓ and higher concentrations. The application factor range (MATC/96-hour LC50) for sheepshead minnows exposed continuously to Kepone is from 0.001 to 0.002. Other adverse effects of Kepone exosure were: external signs of poisioning in fish exposed to concentrations ≥0.074 µg/ℓ, fatty degeneration of the liver and histological evidence of vertebral damage in fish exposed to 0.78 µg/ℓ, reduced growth of parental fish at 0.39 and 0.78 µg/ℓ, reduced fecundity and dertility of eggs at 0.78 µg/ℓ, and reduced survival of embryos produced by fish exposed to 0.78 µg/ℓ. Average bioconcentration factors (concentration in tissue averaged measured concentration in water) were: 21-day juveniles-2,600; 42-day juveniles-2,800, adult males-7,600, adult females-5,700, eggs-3,300, and 28-day juvenile progeny-3,100.

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Effects of Kepone® on the Sheepshead Minnow in an Entire Life-cycle Toxicity Test[1]

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Abstract. A life-cycle toxicity test with the estuarine sheepshead minnow (*Cyprinodon variegatus*) and the insecticide Kepone® (chlordecone) in seawater showed that the maximum acceptable toxicant concentration (MATC) lies between 0.074 and 0.12 μg Kepone/L, based on reduced standard length of progeny at 0.12 μg/L and higher concentrations. The application factor range (MATC/96-hr LC50) for sheepshead minnows exposed continuously to Kepone is from 0.001 to 0.002. Other adverse effects of Kepone exposure were: External signs of poisoning in fish exposed to concentrations ≥0.074 μg/L, fatty degeneration of the liver and histological evidence of vertebral damage in some fish exposed to 0.78 μg/L, reduced growth of parental fish at 0.39 and 0.78 μg/L, reduced fecundity and fertility of eggs at 0.78 μg/L, and reduced survival of embryos produced by fish exposed to 0.78 μg/L. Average bioconcentration factors (concentration in tissue ÷ average measured concentration in water) ranged from 2,600 for 21-day-old juveniles to 7,600 for adult males.

Pollution of the James River estuary, Virginia with the organochlorine insecticide Kepone® (1, 1a, 3, 3a, 4, 5, 5a, 5b, 6-decachloroctahydro-1, 3, 4-metheno-2*H*-cyclobuta(cd)pentalen-2-one) has prompted extensive research on its toxicity to estuarine biota (Bookhout *et al.* 1980; Bourquin *et al.* 1978b; Couch *et al.* 1977; Hansen *et al.* 1977; Nimmo *et al.* 1977; Provenzano *et al.* 1978; Rubinstein 1979; Schimmel and Wilson 1977; Walsh *et al.* 1977) and on bioaccumulation, bioconcentration, transfer, and loss (Bahner and Oglesby 1979; Bahner *et al.* 1977; Drifmeyer *et al.* 1980; Schimmel *et al.* 1979). The fate of Kepone in artificial estuarine ecosystems was investigated by Bourquin *et al.* (1978a) and Garnas *et al.* (1977).

The toxicity of Kepone to estuarine fishes was investigated by Couch *et al.* (1977); Hansen *et al.* (1977); and Schimmel and Wilson (1977) in flow-through tests with measured concentrations. Couch *et al.* (1977) described scoliosis and its histological consequences in sheepshead minnows (*Cyprinodon variegatus*) exposed to four μg Kepone per L of seawater for 10 to 17 days. Schimmel and Wilson (1977) reported 96-hr LC50 values of 69.5 μg/L for sheepshead minnows and 6.6 μg/L for spot (*Leiostomus xanthurus*). Hansen *et al.* (1977) exposed sheepshead minnows to Kepone concentrations that ranged from 0.08 to 33 μg/L in a toxicity test that began with adults that were exposed for four weeks before hormonally enhancing the production of eggs which were then fertilized artificially. They reported a 28-day LC50 of 1.3 μg/L for adults and found that a significant number of embryos from fish exposed to 1.9 μg/L developed abnormally and died, even when incubated in uncontaminated seawater. They also found that the average standard length of juvenile progeny in all Kepone concentrations was significantly less than that of control fish. Buckler (1979) determined acute and chronic toxicities of Kepone to the freshwater fathead minnow (*Pimephales promelas*). He reported a 96-hr LC50 of 340 μg/L, a 96-hr EC50 of 18 μg/L, and a MATC between 1.2 and 3.1 μg/L.

This investigation was conducted to determine the maximum acceptable toxicant concentration (MATC) and application factor for sheepshead minnows continuously exposed to Kepone in an entire life-cycle toxicity test.

Materials and Methods

Seawater from Santa Rosa Sound, FL, was pumped through a sand filter and a 20μ polypropylene filter to a constant-head box

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where it was heated to 30 ± 2°C and aerated. Water (average salinity 24.2‰, range 11 to 31‰) was delivered intermittently to exposure aquaria which contained 70 L by a dosing apparatus similar to that described by Schimmel *et al.* (1974). At each cycle, the dosing apparatus delivered two L of seawater containing the desired Kepone concentration and 8.8 μL of the carrier solvent (triethylene glycol) for each of the seven treatments. Water flow from the dosing apparatus was divided to deliver one L to each duplicate aquarium per treatment. Two water baths, designated upper and lower and each containing one set of duplicate aquaria, maintained the exposure temperature at 30 ± 2°C. The photoperiod was 12 hr light and 12 hr darkness. Nominal Kepone concentrations were control-with-carrier, 0.02, 0.05, 0.10, 0.21, 0.46, and 1.0 μg/L. Future references to Kepone concentrations will be to the average measured concentration (Table 1).

The 141-day life-cycle toxicity test began with artificially spawned embryos from adult sheepshead minnows collected near the laboratory. Adult fish were treated once with 1:4,000 formalin in filtered seawater at 30°C for one hr to remove trematodes during a five-day period prior to hormonal enhancement of egg production by the method of Hansen *et al.* (1973). Eggs from 23 females were incubated for approximately one hr with macerated testes from ten males in approximately 100 ml of 30°C filtered seawater. There were no detectable Kepone or other organochlorine insecticides (<0.02 mg/kg) or PCBs (<0.25 mg/kg) in the eggs. Twenty fertile eggs were placed in each of four incubation cups (9-cm I. D. glass Petri dish bottoms to which 10-cm-high 450μ nylon mesh tubes were attached) per aquarium. Incubation cups were placed in trays (internal dimensions = 36.5 cm × 24.5 cm × 9 cm high) within the aquaria. The trays received water from the dosing apparatus, then delivered it to the aquaria via self-starting siphons that caused the water depth in the trays to fluctuate between 3 and 8 cm.

Embryos and hatched fish were observed daily for mortality and signs of poisoning. On day 21, fish in each incubation cup were photographed for later measurement of standard length (SL). After combining fish from the incubation cups within duplicates, ten fish from each duplicate were removed and pooled within treatments for chemical analysis. After replacing the self-starting siphon with a stand pipe, 42 fish were released into the tray in each duplicate aquarium. Fish were also photographed on days 42 and 64. On day 42, five fish were removed from each duplicate and combined within treatments for chemical analysis; remaining fish were released into the exposure aquaria. Fish ≤42 days old were fed live brine shrimp nauplii (*Artemia* sp.); fish >42 days old were fed frozen brine shrimp. Neither food contained detectable Kepone (<0.03 mg/kg).

The effect of Kepone on sheepshead minnow reproduction was investigated in five spawning trials, each of seven days duration, between exposure days 83 and 131. After fish attained reproductive size (≥26 mm SL), three females and two males were placed in glass spawning chambers (inside dimensions = 34.5 × 19 × 23 cm high; water depth of approximately 15 cm) located in each exposure aquarium. The ends and bottom of the chambers were covered with 6.4 mm² stainless steel screen that allowed eggs to fall onto a removable tray constructed of Plexiglas® and 450μ nylon mesh. Eggs were removed daily, counted, and examined microscopically to determine percentage fertility. All fish from spawning trials 1, 2, 4, and 5 were combined within treatments, then separated by sexes and analyzed for Kepone.

Nine control fish and ten fish exposed to 0.78 μg Kepone/L were preserved, following spawning trial 3, for histological examination. They were fixed in Davidson's fixative, embedded in paraffin, and sectioned in either the longitudinal horizontal plane or in cross section in the region of caudal vertebrae 17–21, cephalic to caudal count.

Table 1. Nominal and average measured Kepone concentrations (μg/L) in weekly water samples during an entire life-cycle toxicity test with the sheepshead minnow (*Cyprinodon variegatus*).

Nominal	Measured		
	N	Mean	SD
Carrier control[a]	18[b]	ND[c]	
0.021	20	ND[d]	
0.046	13[e]	0.041	0.016
0.10	20[f]	0.074	0.051
0.21	21	0.12	0.044
0.46	20[g]	0.39	0.27
1.0	21	0.78	0.36

[a] Contained 4.4 μL triethylene glycol/L.
[b] Only the first sample contained detectable Kepone (0.026 μg/L) and it was probably the result of mislabeling.
[c] ND = not detectable (<0.02 μg/L).
[d] Three of the analyses were detectable and ranged between 0.023 and 0.84 μg/L.
[e] Eight additional analyses were not detectable and are not included in the mean and SD.
[f] Three additional water samples were not detectable and were excluded from the mean and SD.
[g] One additional water sample was not detectable and was excluded from the mean and SD.

The effect of Kepone on progeny of adults that had been exposed continuously from 88 to 113 days was investigated by placing twenty fertile eggs in each of four incubation cups within trays located in the same aquarium in which the eggs were spawned. Extra eggs from the spawning trials were frozen for analysis of Kepone. Embryos and hatched fish were observed daily for death and signs of poisoning. After 28 days, fish were photographed for length measurement, weighed, combined within treatments, and frozen until analyzed for Kepone. Length and weight analyses were performed on progeny that were spawned between days 88 and 96.

Kepone contents of tissues and water were determined by gas chromatographic methods described by Hansen *et al.* (1977). Water from all concentrations was analyzed weekly, samples being taken from each duplicate on alternate weeks. Kepone was purchased from Chem Service, West Chester, PA and was listed as 99% pure; our analyses indicated 88% purity. Dissolved oxygen samples were taken at approximately weekly intervals, beginning on exposure day 27, alternating between duplicate aquaria for each test concentration. Oxygen content in each treatment (Figure 1), determined by the modified Winkler method (Strickland and Parsons 1968), averaged 3.5 to 5.0 mg/L (overall range, 1.0 to 6.8 mg/L).

Analysis of variance and Newman-Keuls method were used to analyze weight data. All other data were analyzed by analysis of variance and Duncan's multiple range test. Percentages were examined both with and without arcsin transformation before analyses. The significance level of $\alpha = 0.05$ was chosen for all statistical analyses.

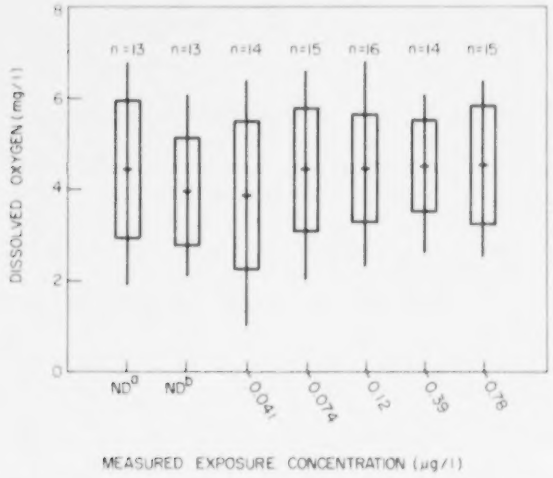

Fig. 1. Dissolved oxygen concentrations in aquaria during 141-day Kepone chronic toxicity test with the sheepshead minnows (– = mean, I = range, □ = standard deviation)
[a] Control with carrier, ND = not detectable (<0.02 µg/L)
[b] Nominal concentration = 0.021 µg/L, ND = not detectable (<0.02 µg/L)

Table 2. Survival of embryos, fry, and juvenile sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to measured concentrations of Kepone for 20 days. Unless otherwise noted, exposure began with 80 embryos obtained from feral fish per treatment duplicate. No statistical difference ($\alpha = 0.05$) was found between treatments

Exposure concentration (µg/L)	Duplicate aquarium	Survival (%)		
		Embryos	Fry and juveniles	Combined
ND[a]	upper	79	90	71
ND[a]	lower	80	91	72
ND[b]	upper	72	89	65
ND[b]	lower	80	100	80
0.041	upper	75	95	71
0.041	lower	80	98	79
0.074	upper	74	95	70
0.074	lower	70	95	66
0.12	upper	79[c]	96	75
0.12	lower	78	98	76
0.39	upper	69	98	68
0.39	lower	84[d]	94	78
0.78	upper	79	95	75
0.78	lower	79	90	71

[a] Control with carrier, ND = not detectable (<0.02 µg/L)
[b] Nominal concentration = 0.021 µg/L, ND = not detectable (<0.02 µg/L)
[c] Seventy-nine embryos
[d] Eighty-one embryos

Results

Survival, Signs of Poisoning, and Pathology of Parental Fish

There was no significant ($\alpha = 0.05$) toxicant-related mortality among parental fish. Overall survival of embryos and hatched fish averaged 72.8% during the 20 days that they were in the incubation cups (Table 2). Excluding accidental and sampling deaths, mortality in individual aquaria from day 21 to the end of the experiment ranged from 0 to approximately 20%, with no obvious concentration dependence. External signs of poisoning were observed in parental fish exposed to Kepone concentrations ≥0.074 µg/L. Darkening of the posterior one-third of the body was observed occasionally in fish exposed to 0.074 µg/L, more frequently in fish exposed to higher concentrations. This condition was first observed on day 32 in fish exposed in 0.78 µg/L (5% incidence on day 39) and on day 42 in fish exposed to 0.074, 0.12, and 0.39 µg Kepone/L. In dorsal view, a majority of the fish exposed to 0.78 µg/L appeared abnormally thickened by day 42. Fish exposed to 0.78 µg Kepone/L frequently appeared lethargic and one individual observed on day 39 had an abnormal lateral flexure of the body. Adult male fish that had been continuously exposed from the zygote stage to 0.39 and 0.78 µg Kepone/L appeared abnormally rotund. The mean ratio of depth to SL of male fish, derived from photographs and preserved specimens, was 0.46 (N = 14) for fish exposed to 0.78 µg/L, 0.42 (N = 5) for fish exposed to 0.39 µg/L, and ranged between 0.35 and 0.39 for the remaining treatments (N = 4 per concentration). This condition was not observed in females.

Of the ten fish exposed to 0.78 µg Kepone/L for 116 days and examined histologically, seven had from moderate to severe fatty degeneration of the liver manifested by large vacuoles in hepatocytes. Several livers had small necrotic foci. Similar nonspecific fatty changes in fish hepatocytes have been reported for fish exposed to certain other organochlorine pesticides (Couch 1975). Although fatty accumulation may occur in relation to diet or stage of growth (*i.e.*, normal physiological feature) in some species of fish, and some fat deposition was found in a few of the control fish, we believe that the more severe fatty change and necrotic foci in the experimental fish was related to Kepone exposure. Sections from three of the fish revealed histologic evidence of vertebral damage (fractured anterior caudal vertebrae with wound repair tissue evident).

These lesions were not observed in the control fish and were suggestive of, but not as severe as, the broken-back-wound-repair injuries observed in the same species following exposure to 4 μg Kepone/L for 10 to 17 days and described in detail by Couch *et al.* (1977).

Growth of Parental Fish

The average standard length of fish continuously exposed from the zygote stage to 0.39 and 0.78 μg Kepone/L for 64 days was significantly shorter ($\alpha = 0.05$) than that of control fish (Table 3). Although other statistically significant differences in length were observed between controls and some of the exposed groups on days 21 and 42, these differences were not consistent between duplicate aquaria and the conclusion was that they were not real.

The shorter standard length of fish continuously exposed to 0.78 μg Kepone/L was particularly obvious when females were selected for the spawning trials between days 83 and 131. Females ≥26 mm SL were usually unavailable from this treatment. Consequently, females in seven of the nine spawning groups selected from 0.78 μg/L averaged less than 26 mm SL (Table 4). Lengths of male fish from the spawning trials were not compared between treatments, because males of adequate length were always available and we biased the selection process by attempting to place females with males of similar length.

Reproduction

Overall egg production in the spawning trials (Table 5) was significantly reduced by exposure to 0.78 μg Kepone/L (48% of control production). The number of eggs per female per day averaged 21.3 for controls and 10.3 for fish exposed to 0.78 μg/L. Although other statistically significant differences in egg production were noted, they were not considered meaningful because they were not significant in both duplicates. Interpretation of these data is difficult, because the females exposed to 0.78 μg Kepone/L and used in the spawning trials were significantly shorter than those in the other treatments and female size was probably a contributing factor in the results obtained in the reproduction study. A significantly ($\alpha = 0.05$) lower percentage of those eggs spawned in 0.78 μg Kepone/L was fertile than in the other treatments (Table 6). The average percentage fertile was 84.5 in 0.78 μg/L and 95.8 in the remaining treatments.

Progeny Survival, Signs of Poisoning, and Growth

Hatching success was significantly diminished in embryos that were spawned and held in 0.78 μg Kepone/L (Table 7). Survival of embryos to hatching in the various treatments ranged from 68% in 0.78 μg/L to 100% in 0.39 μg/L. Survival of fry that hatched was not significantly affected by additional exposure for 28-days post-fertilization. Fry survival ranged from 87.5% in 0.39 μg/L to 97.6 in 0.78 μg/L (Table 7).

Signs of poisoning exhibited by progeny were similar to those observed during the parental exposure. Darkening of the posterior third of the body was observed in at least 5% of the fish in one of the duplicates exposed to 0.39 μg/L on day 22 and intermittently, beginning on day 17, in ≤10% of the fish exposed to 0.78 μg/L. It also became apparent on day 21 that one fish exposed to 0.78 μg/L was abnormally thickened when viewed from above and by day 28, approximately 75% of the fish in this concentration appeared thickened. No signs of poisoning were observed at concentrations ≤0.39 μg/L during the 28-day exposure of progeny.

Length of progeny was significantly affected ($\alpha = 0.05$) by Kepone concentrations ≥0.12 μg/L. Average lengths of fish exposed to 0.12, 0.39, and 0.78 μg/L ranged from 8.9 to 9.5 mm, whereas control animals averaged 10.2 mm (Table 7). Lengths of fish exposed to Kepone concentrations ≤0.074 μg/L were not significantly different from those of controls. These results are consistent with those obtained by Hansen *et al.* (1977) in a similar exposure of progeny of exposed fish. They reported reduced SL of progeny after 36 days exposure to 0.08 μg/L, their lowest Kepone concentration. Our weight data, from total wet weight per incubation cup, reflect the abnormal thickening of fish exposed to 0.78 μg/L and suggest that this condition may have existed in fish exposed to 0.12 and 0.39 μg/L. Even though these three groups were significantly shorter than control animals, their average weights were greater (Table 7); weight of fish exposed to 0.78 μg/L ($\bar{x}$ = 29.2 mg) was significantly ($\alpha = 0.05$) greater than that of controls ($\bar{x}$ = 16.8 mg).

Bioconcentration

Kepone was bioconcentrated by juvenile and adult fish continuously exposed from the embryonic stage and in eggs from exposed fish (Table 8). Bioconcentration factors (concentration in tissue ÷ average concentration measured in water) were not de-

Table 3. Average standard length (rounded to the nearest 0.1 mm) attained by sheepshead minnows continuously exposed to measured concentrations of Kepone. Exposure began with embryos approximately one hr after combining gametes from feral fish

Exposure concentration (μg/L)	Day 21[a]			Day 42[a]			Day 64[b]		
	Upper	Lower	Combined	Upper	Lower	Combined	Upper	Lower	Combined
ND[c]	8.9	9.8	9.4	18.9	19.8	19.4	26.4	25.2	25.8
ND[d]	9.9[e]	9.5	9.7	18.9	18.9	18.9	25.4	25.1	25.2
0.041	9.3	10.1	9.7	18.4	19.1	18.8	25.3	24.5	24.9
0.074	9.2	10.2	9.7	18.2	19.2	18.7	24.9	24.7	24.8
0.12	9.6[e]	10.0	9.8	19.3	19.0	19.2	24.9	24.9	24.9
0.39	9.9[e]	8.8[e]	9.3	19.1	17.9[e]	18.6	24.6	24.4	24.5[e]
0.78	9.3	8.5[e]	8.9	17.6	16.6[e]	17.1	22.5	22.4	22.4[e]

[a] Concentration X duplicate interaction significant; therefore, comparisons were made within duplicates
[b] Since concentration X duplicate interaction was not significant, duplicates were combined before making comparisons
[c] Control-with-carrier, ND = not detectable (<0.02 μg/L)
[d] Nominal concentration = 0.021 μg/L, ND = not detectable (<0.02 μg/L)
[e] Significantly different from control (α = 0.05)

Table 4. Average standard length (mm) of female sheepshead minnows continuously exposed to measured Kepone concentrations and used in spawning trials. Groups were monitored in aquaria located in upper and lower water baths

Exposure concentration (μg/L)	Trial I		Trial II		Trial III		Trial IV		Trial V		X		
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	X[a]
ND[b]	25.5	26.7	28.0	30.3	30.0	30.5	33.0	31.2	32.0	28.7	29.7	29.5	29.6
ND[c]	27.0	26.7	28.0	26.3	28.3	32.2	27.0	32.0	30.0	28.7	28.1[d]	29.2	28.6[d]
0.041	27.5	27.5	28.3	30.5	29.0	29.7	31.2	30.5	31.3	30.3	29.5	29.7	29.6
0.074	31.5	27.7	28.0	28.3	30.3	27.7	33.3	29.7	33.3	27.7	31.3[d]	28.2[d]	29.8
0.12	29.5	29.7	26.7	27.8	31.2	27.8	30.8	29.8	31.0	29.3	29.8	28.9	29.4
0.39	29.5	28.3	29.0	28.0	28.3	27.2	27.7	28.3	28.7	31.7	28.6[d]	28.7[d]	28.7[d]
0.78	25.0	26.5	25.3	24.3	24.2	26.3	22.7	25.0	23.0		24.0[d]	25.5[d]	24.7[d]

[a] Overall treatment mean
[b] Control-with-carrier, ND = not detectable (<0.02 μg/L)
[c] Nominal concentration = 0.021 μg/L, ND = not detectable (<0.02 μg/L)
[d] Significantly different from control (α = 0.05)

Table 5. Average number of eggs per female per day produced by sheepshead minnows continuously exposed to measured concentrations of Kepone. Unless noted otherwise, spawning groups consisted of three females and two males from a given exposure concentration and groups were monitored for seven days in duplicate aquaria in upper and lower water baths

Exposure concentration (μg/L)	Trial I		Trial II		Trial III		Trial IV		Trial V		X		
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	X[a]
ND[b]	18.9	16.1	30.8	33.0	18.0	6.9	22.8	13.1[c]	31.1	22.0	24.3	18.3	21.3
ND[d]	24.2	19.7	28.1	19.4	26.6	38.8	24.3	43.2	42.3	12.4	29.1	26.7[c]	27.9[c]
0.041	23.8	38.2	21.6	28.5[c]	28.5	37.4	21.4	38.1	17.9	18.7	22.6	32.2[c]	27.4[c]
0.074	23.2	20.0	35.0	5.8	37.1	30.7	53.4	15.0	46.2	17.4	39.0[c]	17.8	28.4[c]
0.12	25.7	28.8	18.4	27.7[f]	31.6	13.9	34.8	29.6	21.7	17.3	26.4	23.5	25.0
0.39	23.1	33.0	18.5	17.0	32.9	13.6	21.5	27.6	24.9	21.3	24.2	22.5	23.3
0.78	14.8	8.7	24.4	0.6	14.3	13.7	4.9	4.3[c]	7.1[c]		13.1[c]	6.8[c]	10.3[c]

[a] Overall treatment mean
[b] Control-with-carrier, ND = not detectable (<0.02 μg/L)
[c] Group consisted of two females and three males
[d] Nominal concentration = 0.021 μg/L, ND = not detectable (<0.02 μg/L)
[e] Significantly different from control (α = 0.05)
[f] Group consisted of four females and one male

Table 6. Average fertility (%) of eggs produced by sheepshead minnows continuously exposed to measured concentrations of Kepone. Unless noted otherwise, spawning groups consisted of three females and two males from a given exposure concentration and groups were monitored for seven days in duplicate aquaria in upper and lower water baths

Exposure concentration (μg/L)	Trial I		Trial II		Trial III		Trial IV		Trial V		X		
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	$\bar{X}^a$
ND[b]	97.9	98.5	97.9	97.4	88.0	96.0	99.5	99.7[c]	96.5	99.0	96.0	98.1	97.0
ND[d]	98.7	88.6	96.8	92.1	98.6	91.6	97.8	99.4	95.9	92.3	97.5	92.8	95.1
0.041	96.4	95.2	97.8	93.3[c]	95.4	99.8	97.2	98.9	98.4	89.5	97.0	95.4	96.2
0.074	97.7	95.5	97.5	76.9	97.8	97.7	99.2	98.5	98.7	98.9	98.2	93.5	95.8
0.12	99.2	96.8	97.8	98.6[e]	98.2	76.6	98.0	98.4	93.4	93.9	97.3	92.7	95.1
0.39	97.7	97.0	98.2	82.7	98.1	94.2	97.9	97.5	99.0	93.6	98.2	93.0	95.5
0.78	87.7	96.0	88.2	52.5	91.8	97.4	80.0	70.6[c]	92.0[e]		88.0[f]	78.3[f]	84.5[f]

[a] Overall treatment mean
[b] Control-with-carrier, ND = not detectable (<0.02 μg/L)
[c] Group consisted of two females and three males
[d] Nominal concentration = 0.021 μg/L, ND = not detectable (<0.02 μg/L)
[e] Group consisted of four females and one male
[f] Significantly different from control ($\alpha = 0.05$)

Table 7. Survival of embryos, fry, and juvenile sheepshead minnows (*Cyprinodon variegatus*), average standard length (SL), and weight of juvenile fish continuously exposed from the zygote stage to measured concentrations of Kepone for 28 days. Unless noted otherwise, survival studies began with 80 embryos per concentration, obtained from fish that had been continuously exposed in the respective concentrations

Average exposure concentration (μg/L)	Survival (%)			Growth	
	Embryos	Fry and juveniles	Combined	Average SL (mm)	Average weight (mg)
ND[a]	97.5	97.4	95.0	10.2	16.8
ND[b]	96.2	94.9	91.2	10.5	20.7
0.041	90.1[c]	90.7	82.1	10.3	20.5
0.074	89.0[d]	91.6	82.0	10.2	21.0
0.12	95.0[e]	88.9	84.0	9.5[f]	19.7
0.39	100	87.5	87.5	8.9[f]	19.5
0.78	68.0[e,f]	97.6	66.0[f]	9.4[f]	29.2[f]

[a] Control with carrier, ND = not detectable (<0.02 μg/L)
[b] Nominal concentration = 0.021 μg/L, ND = not detectable (<0.02 μg/L)
[c] One hundred and one embryos
[d] One hundred embryos
[e] Eighty-one embryos
[f] Significantly different from control ($\alpha = 0.05$)

pendent on exposure concentration. Average bioconcentration factors (BCFs) were: 2,600 for 21-day juveniles, 2,800 for 42-day juveniles, 7,600 for adult males, 5,700 for adult females, 3,300 for eggs from the adult females and 3,100 for 28-day juvenile progeny. Schimmel and Wilson (1977) reported average BCFs of 1,548 for sheepshead minnows and 1,221 for spot after 96-hr exposure to Kepone in flowing seawater. Bahner *et al.* (1977) reported a mean BCF of 7,115 for sheepshead minnows continuously exposed to 0.05 μg Kepone/L for 28 days, slightly higher concentrations being found in females than in males. For sheepshead minnows exposed to Kepone at 30°C, Hansen *et al.* (1977) found that BCFs for juveniles, but not adults, were inversely dependent on exposure concentration. The bioconcentration factors for juveniles exposed for 36 days from the zygote stage ranged from 3,600 to 20,000 (average 7,200). If the BCFs for their lowest exposure concentration are not included in the comparison, our BCF data for juvenile progeny ($\bar{x}$ = 3,100) are similar to theirs ($\bar{x}$ = 4,400). The results obtained by Hansen *et al.* (1977) differed somewhat from ours in that Kepone concentrations in their tests were greater in females than in males at the same exposure concentration and concentrations in eggs were similar to those in females. However, Hansen *et al.* (1977) enhanced egg production

Table 8. Concentrations of Kepone (mg/kg, whole body, wet weight) measured in continuously exposed sheepshead minnows (*Cyprinodon variegatus*) and in embryos from exposed females. Bioconcentration factors (BCF = concentration in tissues ÷ average measured concentration in water) are in parentheses

	Average measured exposure concentration (μg/L)					BCF
Life Stage	0.041	0.074	0.12	0.39	0.78	X
Juveniles, day 21	0.074	0.16	0.42	0.87	2.4	
	(1,800)	(2,200)	(3,500)	(2,200)	(3,100)	(2,600)
Juveniles, day 42	0.1	0.15	0.34	0.97	3.2	
	(2,400)	(2,000)	(2,800)	(2,500)	(4,100)	(2,800)
Adult males, days	0.16	0.56	1.2	3.0	6.8	
90-133	(3,900)	(7,600)	(10,000)	(7,700)	(8,700)	(7,600)
Adult females, days	0.15	0.42	0.86	2.2	5.0	
90-133	(3,700)	(5,700)	(7,200)	(5,600)	(6,400)	(5,700)
Embryos, days	0.12	0.25	0.47	1.3	2.3	
85-133	(2,900)	(3,400)	(3,900)	(3,300)	(2,900)	(3,300)
Juvenile progeny,	0.099	0.3	0.41	1.1	2.3	
day 28	(2,400)	(4,100)	(3,400)	(2,800)	(2,900)	(3,100)

with hormones and determined residues in fish after removal of gonads, whereas in our study, eggs were spawned without enhancement and residue analyses were performed on whole fish.

MATC and Application Factor

The maximum acceptable toxicant concentration (MATC) for sheepshead minnows continuously exposed to Kepone, based on reduced length of progeny, is between 0.074 and 0.12 μg/L. This MATC range is supported by the findings of Hansen *et al.* (1977), who reported reduced lengths of sheepshead minnow progeny at 0.08 μg/L, their lowest Kepone concentration. The MATC range for the freshwater fathead minnow is between 1.2 and 3.1 μg Kepone/L (Buckler 1979).

The application factor (MATC ÷ 96-hr LC50) for sheepshead minnows exposed to Kepone is between 0.001 and 0.002. This is similar to an application factor of between 0.004 and 0.009 that can be derived from the data reported by Buckler (1979) for fathead minnows exposed to Kepone.

Discussion

Relatively short-term exposure of sheepshead minnows and fathead minnows to Kepone at concentrations approximately one-tenth of their 96-hr LC50's results in scoliosis or an apparent fracture or dislocation of the vertebral column or both (Couch *et al.* 1977; Hansen *et al.* 1977; Buckler 1979). In the present study, test animals chronically exposed to Kepone at concentrations approximately one-thousandth of the 96-hr LC50 were significantly shorter than control animals. Abnormal rotundity and evidence of vertebral damage occurred in some of the animals at approximately one two-hundredth of the LC50. Buckler (1979) reported reduced growth of fathead minnows exposed chronically to Kepone concentrations at approximately one-hundredth of the 96-hr LC50.

Although comparisons between laboratory toxicity test results and observations in the field are frequently tenuous, it is desirable to attempt it because of the James River Kepone contamination. In the field, deformed animals could be readily detected, but reduced growth would be difficult to decument. The James River area of Virginia is heavily industrialized and vertebral anomalies could be attributed to a variety of causes other than Kepone (Bengsston 1975; Couch *et al.* 1979; Sindermann 1979). Merriner and Chao (1977) reported many deformed spot (*Leiostomus xanthurus*) collected from the James River since 1971 and up to 40% incidence of "stumpy" spot in July 1975. Huggett and Bender (1980) found that Kepone residues in spot from the James River estuary during late summer of 1976 and 1977 approached 1 mg/kg, a residue that approximates those at which chronic effects occurred in both sheepshead minnows (our study) and fathead minnows (Buckler 1979). Schimmel and Wilson (1977) reported that the 96-hr LC50 of Kepone for spot (6.6 μg/L) is approximately one-tenth that for sheepshead minnows (69.5 μg/L). If spot have an application factor similar to those of previously tested fishes, it is possible that reduced growth of this and other fishes with similar sensitivity may have gone undetected.

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